dichlorofluorescein) and their attachment t[illegible]gonucleotides is also described in Fung et al. U.S. Pat. No. 4;855,225.

The above probes are incubated in approximately 5 molar excess of the target polynucleotide ends as follows: the probes are incubated for 60 minutes at 16° C. with 200 traits of T4 DNA ligase and the anchored target polynucleotide in T4 DNA ligase buffer. after washing. the target polynucleotide is then incubated with 100 units T4 polynucleotide kinase in the manufacturer's. recommended buffer for 30 minutes at 37° C., washed. and again incubated for 30 minutes at 16° C. with 200 units of T4 DNA ligase and the anchored target polynucleotide in T4 DNA ligase buffer. Washing is accomplished by successively flowing volumes of wash buffer over the slide. e.g. TE. disclosed in Sambrook et al (cited above). After the cycle of ligation-phosphorylation-ligation and a final washing. the attached microparticles are scanned for the presence of fluorescent label. the positions and characteristics of which are recorded by the scanning system. The labeled target polynucleotide. i.e. the ligated complex. is then incubated with 10 units of Fok I in the manufacturer's recommended buffer for 30 minutes at 37° C., followed by washing in TE. As a result the target polynucleotide is shortened by one nucleotide on each strand and is ready for the next cycle of ligation and cleavage. The process is continued until twenty nucleotides are identified.

APPENDIX I
Exemplary computer program for generating minimally cross hybridizing sets

```
Program minxh
c
c
c
      integer*2 sub1 (6) ,mset1(1000,6) ,mset2(1000,6)
      dimension nbase(6)
c
c
      write(*,*)'ENTER SUBUNIT LENGTH'
      read(*,100)nsub
100   format(i1)
      open(1,file='sub4.dat',form='formatted',status='new')
c
c
      nset=0
      do 7000 m1=1,3
         do 7000 m2=1,3
            do 7000 m3=1,3
               do 7000 m4=1,3
                  sub1(1)=m1
                  sub1(2)=m2
                  sub1(3)=m3
                  sub1(4)=m4
c
c
      ndiff=3
c
c
c           Generate set of subunits differing from
c           sub1 by at least ndiff nucleotides.
c           Save in mset1.
c
c
      jj=1
      do 900 J=1,nsub
900      mset1(1,j)=sub1(j)
c
c
      do 1000 k1=1,3
         do 1000 k2=1,3
            do 1000 k3=1,3
               do 1000 k4=1,3
```

-continued

APPENDIX I
Exemplary computer program for generating minimally cross hybridizing sets

```
c
c
                nbase(1)=k1
                nbase(2)=k2
                nbase(3)=k3
                nbase(4)=k4
c
          n=0
          do 1200 j=1,nsub
            if(sub1(j) .eq.1 .and. nbase(j) .ne.1 .or.
         1    sub1(j) .eq.2 .and. nbase(j) .ne.2 .or.
         3    sub1(j) .eq.3 .and. nbase(j) .ne.3) then
              n=n+1
              endif
 1200         continue
c
c
          if(n.ge.ndiff) then
c
c
c                       If number of mismatches
c                       is greater than or equal
c                       to ndiff then record
c                       subunit in matrix mset
c
            jj=jj+1
            do 1100 i=1,nsub
 1100         mset1(jj,i)=nbase(i)
            endif
c
c
 1000     continue
c
c
            do 1325 j2=1,nsub
            mset2(1,j2)=mset1(1,j2)
 1325       mset2(2,j2)=mset1(2,j2)
c
c
c                       Compare subunit 2 from
c                       mset1 with each successive
c                       subunit in mset1, i.e. 3,
c                       4,5, . . . etc. Save those
c                       with mismatches .ge. ndiff
c                       in matrix mset2 starting at
c                       position 2.
c                         Next transfer contents
c                       of mset2 into mset1 and
c                       start
c                       comparisons again this time
c                       starting with subunit 3.
c                       Continue until all subunits
c                       undergo the comparisons.
c
c
          npass=0
c
c
 1700     continue
          kk=npass+2
          npass=npass+1
c
c
          do 1500 m=npass+2,jj
            n=0
            do 1600 j=1,nsub
              if(mset1(npass+1,j) .eq.1.and.mset1(m,j) .ne.1.or.
         2      mset1(npass+1,j) .eq.2.and.mset1(m,j) .ne.2.or.
         2      mset1(npass+1,j) .eq.3.and.mset1(m,j) .ne.3) then
                n=n+1
                endif
 1600           continue
              if(n.ge.ndiff) then
                  kk=kk+1
                  do 1625 i=1,nsub
 1625               nset2(kk,i)=mset1(m,i)
```

-continued

APPENDIX I
Exemplary computer program for generating minimally cross hybridizing sets

```
            endif
1500        continue
c
c
c                       kk is the number of subunits
c                       stored in mset2
c
c
c                       Transfer contents of mset2
c                       into mset1 for next pass.
c
c
        do 2000 k=1,kk
           do 2000 m=1,nsub
2000          mset1(k, m)=mset2 (k, m)
        if(kk.lt.jj) then
          jj=kk
          goto 1700
          endif
c
c
         nset=nset+1
        write (1,7009)
7009     format(/)
        do 7008 k=1,kk
7008       write(1,7010)(mset1(k,m),m=1,nsub)
7010    format(4i1)
        write(*,*)
        write(*,120) kk,nset
120     format(1x,'Subunits in set=',i5,2x,'Set No=',i5)
```

-continued

APPENDIX I

Exemplary computer program for generating minimally cross hybridizing sets

```
7000    continue
        close(1)
c
c
        end
```

SUB C4